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Professor of Philosophy

REGENERATION OF CRAYFISH APPENDAGES

BY

MARY ISABELLE STEELE, M. A.
Sometime Fellow in Zoology

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A DISSERTATION SUBMITTED TO THE FACULTY OF THE GRADUATE DEPARTMENT OF THE UNIVERSITY OF MISSOURI, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ARTS.

TABLE OF CONTENTS

REGENERATION OF CRAYFISH APPENDAGES.

INTRODUCTION.

The results embodied in this paper were obtained from experiments carried on continuously from October, 1896, to June, 1901. The work was begun under the direction of Professor Howard Ayers in the Zoölogical Laboratory of the University of Missouri, and was completed under Professor George Lefevre.

I am indebted to Professor Ayers for direction and advice during the first three years of the investigation, and to Professor Lefevre for many valuable suggestions afterwards. I also wish to express my appreciation of assistance given me by Professor E. G. Conklin, of the University of Pennsylvania, in making making certain revisions of the work, and by Dr. Charles Thom and Dr. C. M. Jackson in the preparation of material.

NOTE: The publication of the present paper, practically completed in 1901, was at first postponed in order to incorporate in it the results of certain experiments which were at that time still in progress. Since the conclusion of these experiments, circumstances which have been unavoidable have unfortunately made it necessary to further delay the final appearance of the paper. GEORGE LEFEVRE.

I. HISTORICAL SUMMARY.

Regeneration in Crustacea, esecially in the Decapoda, has been the subject of casual and experimental observation for more than two hundred years. Du Tertre's observations (1654) on the crabs of Guadaloupe are the earliest recorded. Although the subject dates back far beyond the beginnings of modern biological science, yet comparatively little work has been done, and our knowledge, beyond the fact that regeneration of lost parts does take place, is still meagre. Du Tertre's observations were merely casual and not at all extended. Regeneration of parts in Crustacea has, however, been studied by Réaumur (1712), Goodsir ('44), Chantran ('73), Brooks ('87), Herrick ('95), Herbst ('96, '99, 1901), and Morgan ('98, 1900).

Réaumur's observations were made upon crayfish, crabs and lobsters, and his general account of the regenerative process in crayfish is especially noteworthy. Réaumur began his experiments at the seacoast upon crabs and lobsters, but the sea either carried away his boxes or filled them with sand, and he then used crayfish with greater success. The following is quoted by Herrick ('95, p. 103), from Réamur's account of his experiments upon crayfish: "I took several of them from which I broke off a leg; placed them in one of the covered boats, which the fishermen call 'boutiques,' in which they keep fish alive. As I did not allow them any food I had reason to suppose that a reproduction would occur in them like that which I had attempted to prove. My expectation was not in vain. At the end of some months I saw, and this without surprise, since I had expected it—I saw, I say, new legs which took the place of the old ones which I had removed; except in size they were exactly like them; they had the same form in all their parts, the same joints, the same movement." Evidently Réaumur is describing

the regenerated appendage after a moult has taken place, for previous to a moult new legs are not exactly like the old ones "except in size;" constrictions merely appear in the place of true joints, and the movements are far from similar to those of a normal leg. It is difficult to understand what difference he expected to find by not feeding the crayfish, for the only probable effect would be to retard the regeneration.

Réaumur also noticed that the length of time required for the production of new appendages was dependent upon a number of conditions. The growth was more rapid in warm than in cold seasons, and when the legs were broken off in winter they did not grow again until summer. He also cut off the "tails" of crayfish, but states that no regeneration took place and that the animals invariably died a few days after the operation. His attempt to explain the regenerative process is one of the earliest explanations offered. He supposed that scattered throughout the limbs are little "eggs" containing the germs of parts of new limbs and that when an appendage is broken off the juices which go to nourish this limb are then directed to the development of the "eggs" containing parts of new limbs. He saw that before such an explanation would be adequate there would have to be more than one "egg" of the same kind, for a regenerated leg can be broken off and another one regenerated in its place. In fact the process can be repeated a number of times, and obviously there would have to be reserve germs for an indefinite number of regenerations. Such an explanation immediately reminds one of Bonnet's theory (1770) of head and tail stuffs and their influence upon germs, except that Bonnet did not suppose specifically different germs at every level. Compare also Weismann's doctrine of determinants.

Chantran ('71) in the study of the growth and development of crayfish incidentally made some observations upon

regeneration. He says that the antennae are regenerated between two successive moults but that other appendages must pass through three moults in order to be regenerated. In a later paper ('73) he gives an account of his experiments upon the eyes of crayfish. He finds that when a part of the eye is removed regeneration takes place more or less rapidly according to the age of the individual and the season of the year. When the eyes are cut off in October at the close of the moulting season no new growth is apparent until the following May. When the whole eye is excised no regeneration takes place.

Goodsir ('80) gives a short account of the regeneration of lost parts in Crustacea, and attempts an explanation of the process, which, however, is not in accordance with results obtained by other observers.

Brooks ('87) confined his observations to the common lobster (*Homarus vulgaris*). His paper is not at hand and I have but a very meagre account of it. Herrick ('95, p. 105) mentions some of Brooks' results. They agree with similar observations made by Herrick himself upon the regenerative processes in the lobster.

Herrick ('95, pp. 104-108) gives a very concise account of his observations upon the regeneration of the chelipeds, antennae and some of the periopods of the lobster. He, too, has noticed that the rate at which regeneration takes place varies greatly with the season of the year, the age of the individual, and whether or not the loss of a limb occurs a short time before or a short time after a moult. In the larvae of lobsters the process of regeneration is very rapid. One case is mentioned in which the cheliped was entirely regenerated within fifteen days. Two moults had occurred, however, during this time. In another case there was complete regeneration after a single moult. But, on the other hand, it may require as long as a month for a young lobster to completely regenerate a lost appendage.

Herrick says that, although so far as known, autotomy does not occur in the antennae, regeneration may take place at any articulation in the flagellum or the stalk. In the young lobster the flagellum of the second antennae may be completely restored without an intervening moult, but in adult individuals one moult at least seems necessary for complete restoration. The internal changes, which do not differ from those observed in crayfish, will be referred to in the discussion of the same phenomena there.

The next experimenter upon regeneration in Crustacea was Herbst. As material for experiment he has used Decapods of at least three different families and two genera of Isopods. His experiments have included the removal of both the whole and a portion of the eye, and have extended over the past five or six years. The first series of experiments was confined to the shrimp, Palaemon ('95), and was primarily undertaken for the purpose of determining whether presence or absence of light has any influence upon regeneration of the eyes. The whole of one or both of the eyes was removed, in most cases only one, for the removal of both eyes was attended with such excessive bleeding that it usually resulted in the almost immediate death of the animal. The experiments were begun in October, but not until the following March was any regeneration apparent. When at last the regenerated structure did appear, it was an antenna-like organ, not an eye. Herbst speaks of these structures as "*antennenähnlichen-Organen.*" They are not alike for all individuals but exhibit two general types, the first, a short horn-like process consisting of at most two segments, and the second, a longer, segmented out-growth resembling the flagellum of an antenna. On the short horn-like processes long segmented setae or hairs were conspicuous, and these Herbst regards as sensory (l. c., p. 507, Figs. 2 and 6). The long an-

tenna-like structures may be subdivided into two classes: in the first a flagellum arises from the distal end of a larger basal portion which resembles the horn-like process of the first type; and in the second a flagellum proceeds, not from the distal end of the basal horn-like portion, but from its side, thus forming a forked structure borne on a common base. The flagellum is not beset with long hairs as in the shorter process, but on the distal borders of the segments are tufts of setae similar to the setae found on the flagella of the antennules. Presence or abscence of light had no apparent effect upon the character of regeneration, although a much larger percentage of the individuals kept in the dark than of those kept in the light showed regenerative power.

Sicyonia sculpta ('96), a member of the same family as *Palaemon,* furnished material for the second series of experiments. These were also confined to the total excision of the eye, and the observations are recorded with much greater precision in this case than in the first. Most of the animals operated upon were about four and a half centimetres in length. The mortality resulting from the operation was not as great as in the cast of *Palaemon,* but the animals were very much harder to feed, and often refused food for days at a time. They were, consequently, less favorable for experiment, and of 85 individuals operated upon during the latter part of October, on the sixteenth of the following April only 6 were living. No signs of regeneration were apparent before the last of January, even though a moult had taken place. Two, three, or perhaps four moults were found to be necessary before any definite structure appeared. Among those that lived until April, only one showed no regeneration whatever. The structures regenerated by *Sicyonia* resembled closely those already described for *Palaemon.* However, in the case of *Sicyonia,* Herbst comes to the conclusion that the antenna-like organs resemble the antennules, first antennae, more nearly than the second antennae.

Decapods belonging to several different genera, to different families even, were used in the third series of experiments ('99), but in all cases the results were similar to those obtained in the previous experiments. But in this latter investigation, the observations extended over a much greater period of time than in the previous ones, and consequently a number of details were brought out here that had not been observed before; for example, Herbst found that the regenerated structure increased in length and in number of segments as the length of time and number of moults increased; and also that the number of rows of sensory hairs, born by the flagella, became more numerous, one case being recorded in which the number of rows of hairs increased between two successive moults from 21 to 33. Several instances are mentioned in which the flagellum was 2.5 to 3 cm. in length and consisted of 110 to 150 segments. Herbst also determined the regenerative capacity of the heteromorphic structure, and found that after removal of either the whole or a part of it a similar structure was again regenerated.

At the close of the third series of experiments, there is given a *résumé* of the chief facts observed up to this time. The main points brought out are as follows: "Experiments upon *Palaemon squilla, P. rectirostris* and *P. serratus, Palaemonetes varians, Sicyonia sculpta, Astacus fluviatilis, Palinurus vulgaris, Scyllarus arctus,* and *Eupagurus psideauxii* show that after total excision of the eye, an eye is never regenerated, but instead, whenever regeneration takes place, there appears a heteromorphic structure which always resembles more or less an antenna. Some portion of this regenerated structure is always provided with long sensory hairs which, in some instances, are developed on both the basal and the distal regions." A point worthy of attention in the consideration of these experiments is the diversity of forms that were used as material for experiment. Individuals belonging to three widely distinct families were used and still the results were in the main identical.

The fourth series of experiments ('99), deals chiefly with results obtained after removal of only a portion of the eye of the stalked-eyed Decapods, *Palaemon* and *Palinurus,* and the entire eye of the sessile-eyed *Porcellana.* The experiments, however, also included the removal of the eyes of the Isopods, *Idothea tricuspidata* and *Sphaeroma serratum,* and *Palinurus vulgaris* and several species of *Palaemon* were used in the removal of only a part of the eye. The cuts removing a part of the eye were made at various angles and at different levels, but in all cases the whole or greater part of the eye-stalk was spared. Results of these experiments differed from previous results in that the regenerated structure resembled not an antenna but an eye, not exactly normal to be sure, but showing a regeneration of pigment and, in some instances at least, the suggestion of ommatidia. *Porcellana,* after total excision of the eye, regenerated not an antenna-like structure but an eye, and indeed in this case the regenerated eye seems to approach very closely to the normal one. The Isopods, *Sphaeroma serratum* and *Idothea tricuspidata,* after the removal of the entire eye, also regenerated eyes.

The results obtained from all of his experiments led Herbst to the following conclusions: first, the presence or absence of the optic ganglion determines whether an eye or an antenna-like organ shall be regenerated; second, when the optic ganglion is present an eye is regenerated; third, removal of the optic ganglion causes an antenna-like organ to be regenerated in place of an eye. In support of the second conclusion is the fact that *Porcellana, Idothea* and *Sphaeroma* regenerate eyes, although the entire eye has been removed. In these sessile-eyed forms, however, the optic ganglion is directly connected with the brain and consequently is not injured even when the eye is wholly removed. Further evidence in favor of this conclusion lies in the fact that in stalked-eyed forms the eye is regenerated after re-

moval of only the upper portion, in which case the optic ganglion has been but little or not at all injured. On the other hand, in support of the third conclusion, is the fact that, after removal of the eye, together with the stalk, and consequently of the entire optic ganglion, the only structure ever regenerated is antenna-like.

Morgan ('98), has made a study of regeneration in the hermit crab, *Eupagurus*. His experiments were made in order to determine whether or not any relation exists between power of regeneration and liability to injury. He first estimated the percentage of appendages lost under natural conditions and then carried on a series of experiments upon the different appendages, both those that were frequently injured and those that were seldom, if ever, injured. The results of his experiments led him to the conclusion that the power of regeneration and the liability to injury are in no way correlated and are not causally connected.

In a second paper, Morgan (:00) deals with the same subject. The hermit crab was again used as material for experiment and the results obtained seem to prove beyond a doubt that, for the hermit crab at least, no relation whatever exists between liability to injury and power of regeneration. It is well known that in cases of autotomy the break occurs between the second and third joints, i. e., between the basipodite and ischiopodite, using Huxley's nomenclature. Normally, of course, regeneration takes place from this point, but he found, however, that regeneration might take place not only at the breaking joint, but also at a joint either proximal or distal to it, and not only at a joint, but also from a cut surface between two joints.

Morgan ('98) also experimented upon the eyes of the hermit crab. When the entire eye was removed, his results were the same as those obtained by Herbst in the similar case. On the other hand, when only a part of the eye is cut off, an eye and not a heteromorphic structure is regenerated.

The general discussion of the theoretical considerations and the criticism of the above-mentioned experiments, together with a consideration of their relation to the results of my own experiments, are to be found in the latter part of this paper.

II. METHODS.

In the fall of 1896, I began a series of experiments and observations upon the regeneration of crayfish of the genus *Cambarus*. Two species were used, *C. virilis* and *C. gracilis*, and since the beginning of my experiments I have continually kept crayfish under observation. Great difficulty has been experienced, however, in keeping the animals alive until the completion of the process of regeneration, and this has, in some degree, interfered with the entire success of individual experiments. After many trials I finally devised, if not an entirely, at least a fairly satisfactory method of taking care of them. Before any definite and conclusive results can be obtained it is necessary that the crayfish should pass through at least one or two moults, for otherwise an appendage is never completely regenerated. Very often the crayfish dies in the act of moulting, or, if it lives through the process, dies soon afterwards.

When I first began the experiments, I kept several crayfish in the same compartment, fed them nearly every day and also changed the water on them daily, never allowing the water to completely cover them. I learned by experience that I could neither keep several together nor feed them each day. When several were placed in the same dish the larger ones would sooner or later eat the smaller ones, or when one moulted it was almost certain to be attacked in its helpless condition and devoured. There were several disadvantages in isolating individual animals, and I therefore tried different schemes for keeping them together. At first they were put into a large wire cage

placed in a pond, as there the conditions were as nearly normal as possible. This method proved unsatisfactory, however, for even when the cage was left undisturbed by intruders, as was not always the case, I had no means of keeping a record of individual crayfish. Keeping them in standing water in the laboratory was attended by one especially objectionable result. A sediment, and in some cases, a fungus growth, appeared all over the animals. I tried various means of removing this, which was particularly troublesome about the eyes, the antennae and the bases of the legs, but the only practicable method was to give them occasionally a thorough scrubbing with a rather stiff brush. Care has to be taken not to break off their legs in this operation. It was practically impossible to keep them in running water, as individual crayfish had to be placed in separate dishes. But the presence of sand or gravel in the vessels seemed to make conditions a little better, although it increased the difficulty of feeding the crayfish.

As was mentioned above, I at first tried feeding them every day, but at last learned that they thrived much better if fed only once or twice a week.

The method I now use is to keep each one in a separate glass dish, with or without gravel in the bottom; nearly cover them with water, changing it every other day, sometimes every day; feed them once or twice a week with fresh meat, occasionally with bread. They are especially fond of fresh liver and of crayfish flesh. The method is not altogether satisfactory, but it yields a fair degree of success.

I further found that the success of an experiment also depended greatly upon the time of year at which the crayfish were operated upon. Those operated upon during the fall and winter seldom live through a moult and often do not moult at the usual season. Apparently their inactivity in the laboratory is not conducive to good health, and since the moult probably does

not occur unless the animal is in its normal physiological condition, few of them are hardy enough to be kept all winter in confinement and still retain sufficient vitality to undergo a moult. My most favorable results, especially in removing the entire eye, have been obtained when crayfish were used soon after having been brought into the laboratory during March and April. These usually moulted within a few weeks and in some cases showed a surprisingly rapid regeneration.

III. OBSERVATIONS.

In my observations and experiments especial attention has been given to the regeneration of the chelipeds, antennae, tail-fins, and the eyes, although experiments upon the ambulatory appendages, swimmerets, gills, and exoskeleton have been carried on to some extent. The greatest part of my observations have been confined to the eyes, but the discussion of the regeneration of these organs will be reserved until the end.

1. CHELIPEDS.

It is well known that crayfish in common with other Decapods practice autotomy, in case of the great chelae at least. The break occurs normally between the basipodite and ischiopodite, i. e., between the second and third segments. The cheliped at this point is very much flattened and there is no movable joint between basipodite and ischiopodite. Apparently a thin cuticular partition is stretched across the appendage at this point and when the limb is broken off this membrane closes the end of the stump and prevents serious bleeding. A clot of blood soon forms and in a short time the membrane turns brown and becomes more or less hardened. The length of time which expires before the bud of a new limb appears depends upon a variety of conditions, such as the time of year, the age of the crayfish, whether or not the break occurs a short time after or a short time before a moult, and upon other conditions both internal and

external. If the crayfish is old and the break does not occur at or a short time after a moult, the new appendage is not likely to appear until after the next moult. In a young crayfish the new limb may appear whether there has been a recent moult or not.

As has been described by Herrick ('95, p. 164) for the lobster, the first external indication of a new appendage is the appearance of a small white papilla on the end of the old stump. The bud is at first semi-transparent white, and, as it grows, constrictions appear which indicate the future joints. There are at first three transverse constrictions, and then on the most distally constricted portion there appears a longitudinal fissure which marks the separation into dactylopodite and propodite. Figs. 1-4, Pl. I, show the appearance of the papilla and its subsequent transformations up to the time of a moult. Fig. 1 shows the stump before the papilla appears; Fig. 2 its first appearance; Fig. 3 the rudiments of four new segments formed, and in Fig. 4 all five new segments are constricted off. After the papilla once appears, if conditions are favorable, the subsequent growth is rather rapid, at least until the confining cuticle has reached the limit of its extensibility. As the limb-rudiment grows it changes color from a somewhat opaque white to a rather translucent white, then to pink, and finally to bright red. Usually all of the constrictions which mark the future segments of the appendages have appeared before the regenerated portion turns red. The cuticle which covers the limb-rudiment soon reaches its limit of extensibility, and no further external increase in size is apparent until after the next moult. The regenerated appendage does not then exceed half an inch in length, but inside the confining cuticle a very much greater growth has taken place, as is manifest as soon as a moult occurs. How great a growth may be compressed within the cuticle is shown by a comparison of Figs. 5 and 6, Pl. II. Fig. 5 shows the old shell

which the crayfish of Fig 6 has just discarded. The right chela, left in the figure, had been broken off and had formed the new growth indicated in the figure at *a*. When the moult took place and the new chela was released from its confining shell, the appendage expanded to the size and proportions shown at *a*, Fig. 6. At the same time it might be well to call attention to some of the other appendages that had been broken off. The first right ambulatory appendage (Fig. 5, *b*) had been broken off between the ischiopodite and the meros. The injury had taken place only a short time before, and, therefore, practically no new growth had appeared, as is shown at *b*, in Fig. 6. Fig. 5 shows a new growth (*c*), which had not yet been constricted into segments; but that segments had been formed inside the integument is seen by reference to Fig 6 (*c*). There the regenerated appendage shows the complete number of segments fully formed and perfectly normal except in size. The left leg of the same pair has made a no less remarkable growth; compare $\dot{c}$ (Fig. 5) and $\dot{c}$ (Fig. 6). The left chela was broken off during the moult. A comparison of Figs. 7 and 8, Pl. II, also shows very clearly that a considerable new growth is confined within the cuticle. Both chelae (Fig. 7) have been regenerated and show that the new growth (*a*) is of sufficiently long standing for the constrictions of the new segments to have been definitely formed. The first pair of legs have also been regenerated and four of the five new segments are apparent externally. In the moulted appendage, however, all five segments are present, *b* (Fig. 8). Practically the same state of affairs may be seen from a comparison of *c, d,* and *f* (Fig. 7), and *c, d,* and *f* (Fig. 8), except that in *c* (Fig. 8) the terminal segment of the new appendage seems to have been broken off at the last moult.

As has been previously mentioned, the length of time which elapses between the loss of an appendage and the appearance of a new one varies greatly in different individuals, and is depend-

ent, in part at least, upon the time of year and the age of the individual.

The following table gives the record of a number of crayfish, none of which exceeds three inches in length, the majority of them being smaller. It can be seen from the table that the time required for regeneration is extremely variable, in some cases all five new segments developing in less time than is required for the appearance of the papilla in other cases.

No.	Appendage removed.	Date.	Regeneration.	Date.	Regeneration.	Date.	Number of days
1	Chela	Mar. 15	Papilla appeared.	Mar. 22	....	....	7 days.......
2	Chela	Apr. 29	....	....	Five new segs...	May 6..	7 days... ....
3	Chela	May 19	....	. ....	Five new segs...	June 12	23 days.......
4	Chela	May 19	. ...	....	Five new segs...	June 12	23 days. .....
5	Chela	June 7	Papilla appeared.	June 14	....	......	7 days ....
6	Chela	June 7	Papilla appeared.	June 14	....	....	7 days........
7	Chela	June 12	Papilla appeared.	June 17	....	....	5 days........
8	Chela	June 12	4 new segments..	June 19	Five new segs...	June 21	7 and 3 days..
9	Chela	June 12	2 new segments..	June 19	Five new segs...	June 21	7 and 3 days .
10	Chela	Apr. 14	Papilla appeared	Apr. 21	Five new segs...	Apr. 26	7 and 5 days..
11	Chela	Apr. 14	Papilla appeared	Apr. 22	Three new segs..	Apr. 26	8 and 5 days..
12	Chela	Apr. 14	Papilla appeared	Apr. 22	Three new segs..	Apr. 26	8 and 5 days..
13	Chela	Oct. 16	Papilla appeared	. ...	Four new segs...	Nov. 16	31 days.......

In No. 2 the chela was broken off in the process of moulting and it will be noticed that regeneration took place very rapidly in this case. The five new segments were formed within a week. In No. 3 the time required to produce the same regeneration was three weeks. Of course a part of this difference may have been due to a difference in the physiological condition of the two animals, but there is no doubt that the fact that the one had just moulted while the other had not is one of the most important factors in the question. In Nos. 10, 11, and 12 a chela was removed at the same time, and No. 10 had moulted a few days before. The papilla appeared a day earlier in the latter case than in the other two, and the growth was more rapid afterwards. After the papilla once appears, it is difficult to see why the growth should not proceed as rapidly in one case as in the other. The difference must be due to physiological causes.

On October 16, 1896, a large crayfish was obtained from

which one chela was missing. The crayfish was kept in the laboratory through the entire winter and spring. On June 9, 1897, it died without having moulted since its capture. Up to this time a new chela had not appeared, but as soon as the animal died the cuticle stretched across the stump was dissected away and immediately there burst forth a regenerated chela in which at least four new segments had been formed (Figs. 9-10, Pl. I.). Fig. 9 shows the old basipodite together with the regenerated portion immediately after the cutting away of the cuticle that kept it so long from appearing. These parts are abnormal in shape and proportion, owing to the very close quarters in which they had been confined. Although the new growth was formed entirely within the old stump, it was covered with a cuticle similar to the covering of a new appendage which appears in the normal manner. In Fig. 10 the structure is seen from the opposite side, after further dissection and after the specimen had been in alcohol over night. That the new growth had been formed for some time was evident from the color, if the color of an appendage which develops normally can be taken as a criterion, for, as was previously mentioned, after a new appendage is old enough to show all the new segments, it changes from white to pink and finally to bright red. Fig. 11 shows a longitudinal section of this rudimentary appendage. Compare general outline with Fig. 10. Around the edges can be seen the cuticle (*cu*), and just beneath this lie numerous nuclei (*a*) and developing muscle fibres which radiate towards the central part of the section. Throughout the limb there is a delicate network of fibres beset with nuclei. The ground substance consists of a non-cellular, granular material, the coagulated plasma (*g*). No well-defined blood channels are present. At *p* a well-marked band of muscle fibres extends nearly across the segment. This perhaps marks the constriction between two segments. At *e* a constriction is present and a band of muscle fibres extends almost to the opposite side. There is very little reason to doubt

that this also marks a constriction where a joint would have been formed. The cleft at the distal end is a very good indication of the separation of propodite and dactylopodite. The distortion which the appendage naturally suffered from its close quarters makes it very difficult to distinguish the different segments, yet the usual differentiation of tissues has taken place, as can readily be seen by reference to Fig. 11, Pl. I. And at any rate, Figs. 9-11, Pl. I, are sufficient to show that, although no regeneration is apparent externally, this is no proof at all that regeneration has not taken place. Fig. 12, Pl. II, shows a part of the tissue from the region *a* (Fig. 11) highly magnified. This tissue consists of broad granular fibers with large nuclei. In Fig. 13, Pl. II, tissue from the region *c* (Fig. 11) is represented. Here the fibres are more definitely formed, and between the two bands of fibres, *m* and *n*, lies a space filled with coagulated plasma.

Little more need be added in regard to regeneration of the ambulatory appendages. The process is essentially the same in them as in the chelipeds, except that very often the regenerating appendage is longer in appearing than in the case of the chelipeds. This, however, does not always hold true, for sometimes the papilla appears within three or four days after the break occurs.

2. TAIL-FINS.

It was formerly supposed that the sixth pair of abdominal appendages which, with the telson, make up the tail-fin, does not regenerate when removed. Herrick ('95, p. 104) mentions that lobsters are sometimes caught in which the tail-fin and even one or two of the posterior abdominal segments are missing. None of these lobsters ever show any signs of regeneration. Morgan ('98, p. 294), however, finds that in the hermit crab the sixth abdominal appendages do regenerate.

In my experiments upon these appendages in the crayfish I have not been very successful. In several cases individuals experimented upon did not live long enough to show any signs of regeneration. However, among specimens brought into the laboratory I have found several regenerating these appendages. On November 21, 1898, I obtained an individual which had lost the entire left, sixth abdominal appendage, but at the time I found the crayfish, regeneration seemed to have advanced as far as would be expected before a moult. The moult took place on the twenty-first of the following April, and at that time the appendage expanded to three-fourths the size of its fellow of the right side, and in every respect was perfectly normal, its articulations and setae being complete. Compare Figs. 14 and 15, Pl. I. Fig. 14 shows the tail-fin with regenerated left appendage before the moult had taken place, and Fig. 15 the same after the moult.

Several other similar instances have come under my notice during the course of my observations. I recently found in a collection of *C. gracilis* a small crayfish, about 20 mm. in length, that had lost almost the entire telson, yet a new and apparently perfectly normal one was being regenerated (Figs. 16-17, Pl. I). Fig. 16 shows a dorsal view of this regenerating telson enlarged ten times. Evidently no moult had taken place since the injury, for the articulation between proximal and distal segments is only partially outlined, and only a few irregular, short setae are present. The new telson is not perfectly symmetrical. A ventral view is shown in Fig. 17. The indications are that the entire telson had been removed, yet the regenerated anal orifice is perfectly normal, except that it is somewhat nearer the posterior end than in an uninjured telson.

3. SWIMMERETS.

My experiments upon the swimmerets have not been very extended, but I have found none to regenerate except the first pair in the male, which are modified as accessory reproductive

organs. In the case of the other abdominal appendages, except the sixth pair, regeneration, if it does take place, is very slow in beginning. Morgan ('98, p. 294) showed that in the hermit crab a very small percentage of these appendages regenerate, and that the process is very slow in beginning. He suggests that the difference between the rate of regeneration in the abdominal and thoracic legs may be partly due to the difference in food-supply which the two sets of appendages receive.

4. ANTENNAE.

In regard to the regeneration of the antennae I can add little to the observations of others on the lobster, the hermit crab, and other Decapoda. The process is essentially the same in the crayfish as has been described by Herrick ('95, p. 106) for the lobster, except that, according to my observations, regeneration does not take place at different levels along the flagellum. In no case that has come under my notice has regeneration of the flagellum taken place except from the base. In cases where only a part of the flagellum had been removed, I have never observed any indications of regeneration. I have never experimented, however, upon very young individuals, and it may take place in them.

When the antenna is broken off at the base of the flagellum, the regenerated portion first appears as a papilla or bud; then, as it grows, it becomes coiled back upon itself and, after a moult, assumes a normal appearance, except in size. Fig. 18, Pl. I, shows a dorsal view of a regenerated right antenna. No apparent increase in size will take place until after the next moult, for the new portion has already become red.

5. GILLS AND EXOSKELETON.

At the beginning of my work I tried a few experiments upon the gills and exoskeleton. The results of these were,

however, only negative. In several crayfish in which the branchiostegite was cut away, the cut edge seemed to heal over, but new setae did not develop. None of the individuals, however, lived through a moult, and it can not be told with certainty how much regeneration actually took place. In other crayfish the carapace was removed from over the pericardial chamber, but no indication of real regeneration was obtained, even when the hypodermis was left uninjured and only the hardened shell removed; but here again I have never succeeded in keeping the individual operated upon alive for more than two or three weeks. In a number of crayfish a part of the exoskeleton was removed from between the sterna and the abdomen. The wound healed over in these cases and left an enlarged knob of tissue covered by a brown cuticle.

My experiments in removing the gills were never successful. In order to observe the gills is was necessary to cut away part of the carapace overhanging the gill chamber, and crayfish operated upon in this way soon died.

6. REGENERATION OF APPENDAGES FROM LEVELS OTHER THAN THE SECOND JOINT.

It very seldom happens that appendages are broken at any joint except the one between the basipodite and the ischiopodite. This is true not only of the chelipeds but also of the other appendages. Occasionally, however, a crayfish is found in which a leg has been broken off at some other level. If this happens in the chelipeds, the limb is usually thrown off later at the regular breaking-joint, unless the injury occurs in the last one or two segments. Then, no regeneration appears to take place and the wound merely heals over, but at the next moult the appendage is restored to a normal condition. I have only observed one instance of the cheliped regenerating from any level other than the breaking-joint. In this case the regeneration was taking place from the distal end of the meros.

In the ambulatory appendages it is not so unusual to find them broken at different levels and regeneration from these levels taking place. Fig. 19, Pl. I, shows a ventral view of the regenerating right last leg of *C. gracilis,* 35 mm. in length. This appendage was broken off between the third and fourth segments. The new portion is three days old.

I have never known of a single instance occurring under natural conditions, where an appendage was broken off at a level *proximal* to the breaking-joint. My experiments with appendages removed below the second joint have never yielded positive results. New tissue is formed, but in no case has it ever assumed the appearance of a regenerating appendage. I have never seen an individual moult, however, after an appendage had been removed below the usual breaking-joint. I can not speak conclusively on this point.

Morgan (:00, pp. 2 and 3) states that in the hermit crab regeneration takes place very readily at a point proximal to the breaking-joint. In the crayfish, however, it is at least certain that regeneration does not occur as readily, or in the usual manner, proximal to the breaking-joint as at that point or distal to it.

In a collection of crayfish I once found an individual with a double chela (Fig. 20, Pl. I). This chela was evidently a regenerated one, for it was very much smaller than the one on the opposite side and was of less than the normal size for the crayfish which bore it. The two segments proximal to the breaking-joint were of the same size as the corresponding segments on the opposite side, thus indicating that the break had taken place at the usual level.

Deformed claws in lobsters seems to be a much more usual occurrence than in crayfish. Herrick ('95, pp. 144-148) gives some interesting notes on this phenomenon, and also mentions the fact that these deformities have attracted the attention of naturalists for more than two hundred years. Recent writers

who have given special attention to the subject are Faxon ('81) and Bateson ('94). Bateson (l. c., p. 537), reports a crayfish with three extra claws. Among thousands of crayfish that have passed through my hands I have not found as many as a dozen that presented any sort of deformity. At all events the species of crayfish with which I am most familiar, viz., *Cambarus gracilis, C. virilis, C. immunis,* and *C. rusticus* are unusually free from deformities of any kind.

7. EYES.

As was mentioned in the historical summary, some earlier observations have been made upon the regeneration of the eyes of Decapods. Herbst, however, is the only experimenter who has given any special attention to the subject. The results of his work have already been discussed (pp. 5-9). Chantran ('73) also gives a brief account of the process, but I have been unable to procure his original paper. Morgan ('98) discusses very briefly the regeneration of the eyes of the hermit crab. It seems at least certain from Herbst's and Morgan's work that crabs and shrimps are much more favorable forms for experiments than crayfish. From their accounts I should judge that regeneration takes place much more rapidly, in the hermit crabs at least, than in crayfish; and also that there is not nearly so much difficulty in keeping them alive as I have experienced in the case of crayfish. Besides the usual difficulties which have already been mentioned, a large percentage of the individuals whose eyes had been removed, died shortly after the operation. Death, in these cases, I attributed to loss of blood and nervous shock. Fewer died when only the corneal part of the eye was cut off than when the whole stalk was removed, and small individuals usually seemed more hardy than larger ones. The point of paramount importance here, as in the case of the appendages, is to keep the crayfish alive through at least one moult after the removal of the eye.

This was also noted by Chantran who found that nothing definite could be determined concerning the regeneration previous to a moult.

In the case of the hermit crabs, experimented upon by Morgan, apparently a moult is not necessary in order that the regeneration of the eye should become evident. At least no mention is made of a moult having taken place. In his first series of experiments, Herbst does not mention the occurrence of a moult, but in his later work he distinctly states, for *Sicyonia sculpta* at least, that two or three moults must take place before any distinct structure appears. He also mentions moults as having occurred in a number of other instances. In the hermit crab Morgan found the regenerative processes to go on very rapidly, for his experiments were begun August 18 and brought to a close September 15 ('98, pp. 294-5). In cases in which only the top of the eye was removed, less than a month was required for the regeneration of a new pigment spot, and Morgan states that in some instances the pigment body was rounded and sharply defined. Eyes of this sort when sectioned showed that the essential eye structures were being regenerated. As no figures of such sections are shown I am unable to judge of the progress the regenerating parts had made. It scarcely seems possible that sufficient regeneration could have taken place, in so short a length of time, to enable one to form any definite conclusions as to what the final outcome would be. As was mentioned before, Morgan found that, after removal of the entire eye, an antenna-like structure instead of an eye was regenerated. The results of Herbst and Morgan differ in the fact that the regeneration was more rapid, and a larger percentage of the individuals regenerated, in the case of the hermit crabs than in that of the shrimps. This, however, may have been largely due to the difference in the season of the year. Most of Herbst's experiments were begun late in the fall or during the winter, while Morgan's were carried

on during the summer. Morgan's entire series of experiments was begun and ended within a month, while in no case does Herbst report any definite regeneration to have appeared within less than three months, and then not until after two or three moults had taken place.

In my experiments upon crayfish it has proved absolutely true that nothing definite concerning the regeneration can be ascertained until after a moult, although the length of time between the operation and the appearance of the regenerated structure varies in individual cases very greatly. It is true that, when only the distal portion of the eye is removed, an apparent regeneration takes place in a very short time, but it is only a partial restoration in size and not a true functional regeneration. This point will be referred to again and more carefully considered.

The eyes of crayfish have been operated upon at three different levels and at varying angles. First, a part of the cornea, sometimes just the top, was cut away, and sometimes a portion was cut from one side; second, the entire corneal surface was removed; and, third, the whole or the greater part of the optic peduncle was cut away. In the latter case of course, the optic ganglion was either wholly or partially removed.

The results obtained from these experiments will be discussed under two main headings; first, regeneration after removal of a portion of the eye; and second, regeneration after removal of the entire eye. In all cases only one eye was operated upon. I am unable to say how large a percentage of the individuals used in each case ever yielded results of value. Many of them died before reaching the moulting period, and consequently I found it impracticable to keep a record of any except those that were killed for examination or those that exhibited noticeable regeneration before dying. Owing to the rapid disintegration of the tissues no satisfactory results could be obtained from

an internal examination of the regenerated eyes of individuals that had died. In every case I have kept a record of the time occupied by the experiment and of the season of the year. With these preliminary statements, which apply equally to both cases, I shall proceed to the discussion of observations and results.

(*a*) *Regeneration After Removal of a Portion of the Eye.* Under this heading are included the cases in which a part of the cornea and those in which the entire cornea has been removed. Removal of the whole or a portion of the cornea is always attended with more or less bleeding, but a clot of blood is soon formed. Within half an hour after the operation the inner tissues bulge out above the level of the cut edges. The swollen appearance persists for a few days, and in the meantime a brownish crust is formed over the cut surface. This gives the appearance of a very rapid regeneration, while in fact practically none has taken place. Later the swollen end seems to retract, and externally regeneration does not appear as advanced as before. The swelling and subsequent contraction may give rise to the extremely irregular contour which a regenerated eye usually presents. From now on an actual growth takes place and within a few weeks or months, as the case may be, the eye attains a size approximately equal to that of the uninjured one. One specimen in which the top of the eye had been removed on October 29 had apparently undergone a very considerable regeneration by November 10; by the eighteenth of November the eye had regained almost its full size. A casual observation showed no particular difference between this eye and the normal one, except in color. The regenerating eye was brown instead of nearly black, the color of the uninjured eye. Examination of the new growth with a lens, however, showed immediately that no corneal facets had been formed, and, since neither corneal facets nor retinular pigment had been developed, it can not be said that there was

any indication of a true eye present. Sections of an eye in this condition show no well defined structures distal to the optic ganglion except the lamellated cuticle (Fig. 21, Pl. III).

Another conspicuous difference between a normal eye and a regenerating one is the rough jagged surface that the regenerating eye presents. Such an eye lacks the symmetrical form of an uninjured one. I have always attributed these irregular outlines chiefly to the crushing and cutting of the eye when it was operated upon. After a moult the eye is often more regular and may approach very nearly the normal shape, but in practically every case, it is more or less misshapen. Sometimes the new portion seems to be developed mainly on one side; sometimes the top of the eye presents, instead of a smooth convex surface, a rough and wrinkled one. Sections of these eyes show several very interesting points, but fail to prove with certainty that eyes in any true sense are being regenerated, that is, eyes capable of functioning as organs of sight. The condition of these regenerating eyes is made plain by an examination of Figs. 21-23, Pl. III. Fig. 21 shows a section through the central portion of an eye from which the cornea had been removed forty-four days before. The points *a* and *b* mark the limits of the regenerated cuticle. On the left side the line of union extends from *a* to *á*, but on the right the break at *b* is abrupt. The newly developed cuticle is very much less compact than the old cuticle, but is of greater depth. In this instance the irregularity of the surface, of which mention has been made, is caused by the cuticular growth. The remnants of the old crystalline cones (*cr.*) are being cast off by the new cuticle which is developing under them. The section passes through the optic nerve (*op.*) and through the optic ganglion (*gl.*). Just beneath the cuticle is seen a fairly complete row of cells with well defined nuclei (*hy*), which I take to be the hypodermis from which the new cuticle is being secreted.

It is noticeable that there is no such well-defined row of cells along the sides beneath the old cuticle (*o. c.*). At each side and above the distal segment of the optic ganglion, is a very conspicuous, loose network of connective-tissue fibres (*cc.*) which is the characteristic tissue formed in the regenerating eye. At each side are bands of muscle (*m.*) belonging to the eye-stalk. In the upper right-hand portion of the figure are shown several small masses of pigment granules, which have no definite size, shape or arrangement and are probably a part of the pigment of the old retina that had not been entirely removed. Although undoubtedly new tissue has been formed, there is nothing to indicate that a functional eye is being regenerated.

In Fig. 22 is seen a regenerating eye that is even more abnormal in shape than the one shown in the preceding figure. Here the irregularity of outline is not due to unequal development of the cuticle, but of the tissues beneath the cuticle. The regenerated portion of the cuticle extends from *a* to *b* and presents, instead of a smooth convex outline, a very much convoluted one. Even the lower, old portion of the eye appears to have suffered considerable distortion. In the depression at *d* and around the outer surface of the new cuticle is a granular aggregation which probably is partly a mucous secretion from the glands beneath the cuticle, and partly degeneration products, together with an admixture of bacteria. At *ba* there are numerous small, ill-defined bodies which can only be interpreted as being a bacterial zoögloea. In this section the hypodermis forms a continuous layer beneath both old and new portions of the cuticle. Besides the optic nerve (*op.*) and ganglion (*gl.*) very little internal structure is to be seen, except the loose connective tissue network. To the right at *m*, are some small muscle bands and just internal to these is a well defined connective tissue band. As in the preceding figure, there is nothing to indicate that the ommatidia are being regenerated.

Fig. 23 shows still another abnormality in the growth and development of the cuticle. Here only the calcified cuticle and underlying hypodermis are shown. The internal structure is practically identical with that of the preceding figures. In this case only one side of the corneal surface had been removed; an oblique cut passing along the line *ab* removed perhaps a fourth of it. The regenerating tissues are but ten days old. The hypodermis is a continuous layer beneath both old and new cuticle. On the right the cuticle has grown inwards a considerable distance; or probably it would be more correct to suppose that the cuticle had formed from *b* over to *a* before an appreciable amount of tissue had been developed; then, the internal tissues growing rapidly forced the hypodermis and cuticle back, so that an apparent invagination of the cuticle was produced. There have been, it seems, two periods of activity in the secretion of the new cuticle. The outer, older portion (*n. c.*) seems to be more compact than the younger, inner part (*n. c̄.*). The inner layers of the new cuticle, together with the hypodermis form a continuous structure beneath both the old cuticle and the older part of the new. From *a* to *d* are what appear to be some remnants of the old corneal facets which are being thrown off.

Fig. 24, Pl. III, is a section of an eye that had been operated upon forty-four days before. Internally it shows the same structures as have been described in previous figures. However, this section is taken eccentrically and shows none of the optic nerve and but little of the optic ganglion. The only new feature in the section is the conspicuous difference between the old and new cuticle. The old cuticle (*o. c.*) shows many more lamellae and those lamellae much more compactly arranged than in the new cuticle (*n. c.*). On the right side, the union of the old and new cuticle at *a* is rough and uneven and less perfect than at *b*.

In only one instance have I ever found any indication of

regenerating corneal facets. A crayfish, from which a part of the eye had been removed, moulted, and a week or ten days later died. The tissues were of course in such a condition that they could not be used for histological purposes, but the cuticular portion was stripped off and examined and the softer tissues beneath teased out and examined under a microscope. The inner tissues showed no sign of either ommatidia or pigment, but a part of the cuticular covering appeared to be developing irregular corneal facets. To be sure, without more evidence than this it is impossible to say whether or not the eye is really regenerated. A single instance in which the parts removed are wholly restored is sufficient to prove that functional regeneration may take place, but as yet this single instance is lacking.

However, at the present writing, May 3, 1901, a small crayfish from which a part of the cornea had been removed on April 9, moulted. Externally, the regenerated eye presents a more normal form than any I have as yet seen, but no corneal facets are visible and the eye is not as dark in color as normally. It has been fixed in Flemming's fluid preparatory to sectioning. Judging from the exterior, this is the most favorable material of the sort that I have had. Fig. 26, Pl. IV, shows an external view of the eye. The part of the cornea removed was taken somewhat to one side rather than at the distal end. This is still evident in the regenerated eye from the apparent encroachment of the stalk portion upon the corneal portion shown at *c*. Otherwise, the eye has much the same appearance as the normal one, except that the corneal portion is relatively much smaller.

Since writing the above the eye shown in Fig. 26 has been sectioned and another crayfish in which the entire cornea had been removed has moulted (Fig. 35, Pl. IV). Both eyes of the latter have been sectioned. Sections of the eye, shown in Fig. 26, Pl. IV, disclose several interesting features. On one side and

through the center of the eye unmistakable ommatidia are present, presumably those belonging to the part of the cornea that had not been removed. Fig. 26 shows the cornea to be almost normal in shape and position, although much reduced in size. It is significant that there is no indication that the injured optic ganglion is being regenerated, and also that the cuticle is just as heavily developed over the corneal portion as over the stalk portion of the eye. But perhaps the most striking feature is what seems to be an increase of muscle tissue along one side and reaching almost to the top of the eye. Fig. 26 a, Pl. III, shows a section taken eccentrically and from the side which is almost destitute of ommatidial structure. Almost the entire interior of the section is taken up by bands of muscle fibres (*m*). To the right, there is still the remains of the optic ganglion (*gl*) and around the periphery are loose connective tissue fibres (*c. c.*); inside the cuticle is a well defined hypodermis, and in the upper part of the figure the irregular remains of some of the crystalline cones. The large amount of muscle tissue can scarcely be explained as due to the plane in which the section chanced to be taken; for the normal eye of the opposite side was cut in connection with the regenerated one, and shows no such preponderance of muscle. Besides, among the large number of normal eyes that I have sectioned in different planes, none show any such development of muscle. It seems evident that more than the normal amount of muscle tissue is developing here at the expense of other tissues. Although only twenty-four days have elapsed since the removal of the part of the eye, new muscle tissue has completely developed. This may be seen by reference to Fig. 41, Pl. IV, which shows some highly magnified muscle fibres taken from region *a*, Fig. 26 a, Pl. III.

Fig. 35, Pl. IV, shows both normal and regenerating eyes of the crayfish referred to above. The entire cornea was

removed April 9, 1901, and the crayfish moulted about six weeks later. The great difference in size between the regenerating eye and the normal one, and the evident pigment spot at the distal end of the former, are noteworthy. Figs. 36 and 37, Pl. II, shows sections of the regenerating eye. In Fig. 36, merely the cuticular outline, the hypodermis, and the arrangement of the pigmented portion at the distal end are seen. The pigment shows a somewhat systematic arrangement, as if ommatidia were being developed. But the pigmented portion lies close under the cuticle. There is nothing to indicate that crystalline cones or corneal facets are being regenerated. The cuticle is equally as dense and thick over the pigmented portion as over the remainder of the eye, and it is very noticeably thicker than the cuticle over the corneal portion of the normal eye. Fig. 37 shows the same features as Fig. 36, and in addition, a part of the optic ganglion (*gl.*). From this figure it will be seen that there is no continuity between the optic ganglion and the pigmented structures, the latter being fairly well separated from the deeper parts by a band of connective tissue. The position of the muscle fibres (*m*) would seem to indicate that the connection between the peripheral eye structures and the optic ganglion is being severed. The ganglion-cells themselves have not the exact appearance of those of the opposite, normal eye. Their outlines are not quite so clearly defined and they are rather more irregular in size. Yet the difference is not clearly enough marked to enable me to decide whether or not the ganglion-cells of the regenerating eye are actually degenerating. But at all events, an examination of the entire series of sections gives no evidence whatever that regeneration of the nervous portion of the eye has taken place.

From the evidence presented by the last two specimens it is hardly possible to conclude that a functional eye has been regenerated. On the one hand, the development of pigment has taken

place, and the pigment-cells approach, to a certain extent, the normal arrangement. On the other hand, however, there is no indication at all that the optic ganglion, the crystalline cones and corneal facets are regenerated; and, furthermore, the cuticle over the pigmented region is as thick as it is over the stalk. It is true that, when only a part of the cornea is removed, as in the case cited on pp. 29-30, the remaining portion may continue to be entirely functional, but here the connection between the ganglion and the ommatidial region has not been completely severed.

(b) *Regeneration After Removal of the Entire Eye.*— This portion of the subject includes, perhaps, the most interesting phase of the question. There are in these experiments results which are at least definite, even though but little understood. However, they are not more inexplicable than other heteromorphic structures.

Whenever the entire eye, together with the whole or greater part of the eye-stalk, is removed, *the eye as an organ of sight is never regenerated.* A new structure may, and in many cases, does develop, but instead of an eye it is a different kind of appendage. Usually this structure is jointed, sometimes like the flagellum of an antenna, but in other cases a pair of flagella like those of the antennules are formed; and again the structure may have no resemblance whatever to a normal appendage but consists of a short two or three-jointed structure similar neither to the eye nor to any appendage the crayfish possesses. The last mentioned structure resembles the appendage that Herbst (loc. cit., pp. 545-6) designated as horn-like (*hornähnlichen*). In discussing the results obtained after removal of the entire eye, I shall refer to a series of crayfish which illustrate the different forms that the regenerated structure may assume.

Type I: *A pair of flagella regenerated.* The first instance observed of the development of these peculiar heteromorphic

structures were in two individuals (*C. virilis*) of medium size from which the entire right eye had been removed on March 15. One of them moulted April 9, the other April 15. At the time of this moult neither of the crayfish showed any indication of a new structure. The individual that moulted April 15, underwent a second moult on May 24, and at this time a well-developed antenna-like structure made its appearance. The crayfish also that moulted April 9 moulted a second time on May 25, and again the same kind of segmented structure appeared. In each case the appendage consisted of a pair of flagella, each of which was composed of a number of segments. The flagella did not project directly outward but curved slightly forward. The inner or anterior flagellum was smaller and shorter than the outer, posterior one. The flagella on the first individual were longer and slenderer than those upon the other one. Unfortunately, however, I lost the specimen before a drawing had been made of this interesting structure. Fig. 27, Pl. IV, shows the shorter, blunter antenna-like product of regeneration in the second crayfish, together with the eye on the opposite side. There is no enlarged segmented base, as in the normal antenna or antennule, but only a slightly enlarged and rounded elevation from which the flagella arise. No conspicuous setae are developed upon them.

After obtaining these two specimens a number of other crayfish were operated upon without any definite results, the trouble being that most of them died before a moult occurred. It may be said, however, that a majority of those that died without moulting were from among the number that were operated upon in the fall and were kept in the laboratory all winter.

Type II: *A single flagellum regenerated.* Besides the two crayfish discussed under Type I, I have obtained but two or three specimens that showed similar structures; and in these

instances a single flagellum and not a pair of flagella was developed. These crayfish were smaller than those mentioned under Type I and all belonged to *C. gracilis* instead of *C. virilis*. The regenerated structure of Type II is represented in Fig. 33, Pl. IV. The crayfish from which this figure was obtained had the entire left eye removed on April 21, shortly after the animal was brought into the laboratory. On June 19 it moulted and at that time there appeared a segmented structure similar to an ordinary flagellum. The flagellum consisted of fourteen segments springing from a slightly elevated base. Before the moult occurred the structure was coiled back upon itself just as in the regenerating flagellum of an antenna. Even after the moult, it did not uncoil immediately. Fig. 33 shows the flagellum after uncoiling and in its relation to the antenna and antennule. The flagellum from the eye-stump curves upward towards the rostrum and is longer than the rostrum; compared with the flagellum of the antennule it is shorter and thicker. The other appendages of this type showed no features not seen in this, except that in one case, at least, the regenerated flagellum did not possess as many segments as are represented in Fig. 33. A shorter time, however, had elapsed between the removal of the eye and the occurrence of the moult.

Type III: *Segmented horn-like structures.* A number of crayfish from which the entire eye had been removed showed, after moulting, not an antenna-like structure, as in the previous cases, but a short horn-like growth which consisted of two, three, or even as many as five or six segments. Types of this structure are shown in Figs. 31, 32, 34, Pl. IV. Fig. 3 (*n*) shows a regenerated appendage consisting of but two segments besides the rounded base; the distal segment is relatively very much smaller than the proximal one. The segments are not movably articulated and the whole structure is covered with a dense hard cut-

icle. The horn-like appendage is shown from the side together with the antenna (*at*) and antennule (*an*) from the same side. The rostrum has been cut away so as to better expose the regenerated part. A similar type of structure is indicated in Fig. 32 at *n*. Here, however, there are three segments and the rounded base, and the arrangement of the segments gives the appendage the appearance of having been spirally twisted. As in the preceding case, the whole appendage is covered with a dense cuticle and possesses no movable articulations. The regenerated structure shown in Fig. 34 (*n*) consists of six segments but lacks the rounded elevated base. In this case, the appendage curves upward and then downward, at the same time twisting inward, towards the median line. In this figure, the antennule is not shown. There is a noticeable difference between these appendages of the crayfish and the somewhat similar structures described by Herbst for the shrimp in the absence of long, sensory hairs in the former. It will be remembered that the *hornähnlichen* processes described by Herbst, were thickly beset on the ends and ventral sides by long segmented setae, considered by him to be of a sensory nature. He seems to imply, although he does not definitely state it, that these, in a measure at least, compensate for the loss of the eye. In the case of the horn-like structures in the crayfish, no setae whatever are present and there is no external indication at all that they are more sensitive than a leg or maxilliped.

Type IV: *Unsegmented horn-like structures.* The chief difference between this type of regenerated appendage and Type III consists in the fact that here only one segment is regenerated. Fig. 30, Pl. IV, shows a regenerated appendage of this type. The crayfish which furnished this particular instance was a small individual of *C. gracilis*. On May 9, the entire eye was removed; the individual afterwards moulted on May 18 and

again on August 18 and finally died on August 20. There is no external indication whatever that the regenerated structure, Fig. 30 (*n*), is segmented. The basal portion (*b*) represents merely a part of the connection between the regenerated structure and the eye on the opposite side. There is little ground for supposing that, had the individual lived longer, the appendage would have become segmented; for more than three months had elapsed between the removal of the eye and the death of the crayfish and two moults had intervened. This is nearly twice as long as the period occupied in the development of the appendage described under Type II (Fig. 33, Pl. IV), and in the latter case fourteen segments were regenerated.

The horn-like processes of Types III and IV agree in the fact that in all instances the process is directed somewhat forward and is usually curved upward and at the same time slightly inward toward the median line. They further agree in the presence of a hard chitinous cuticle and in the absence of setae.

Type V: *Indefinite structure regenerated.* Finally there remain to be considered instances in which no definite regeneration takes place. The number of individuals, which may be considered as belonging to this negative type, are numerous. In the majority of such instances, however, the crayfish died before the occurrence of a moult, or the moult occurred in a very short time after removal of the eye. It is, therefore, impossible to say what the final outcome might have been. In most cases in which the entire eye had been removed, there were no external indications whatever of any regeneration until after a moult, but this is not true of every instance. Fig. 28, Pl. IV, shows the normal left eye and the stump from which the right eye was removed four months before. The animal died without having moulted after the operation. The new tissue that had been formed is represented by the shaded portion distal to the line

c. There are no external signs of differentiation, as constrictions or folds in the tissue, and the cuticle is not even hardened. Apparently nothing is present but a knob of indifferent tissue. Sections of this knob show nothing but a cuticle consisting of numerous lamellae and a small amount of connective tissue. In Fig. 29, Pl. IV, the uninjured left eye and the stump of the right eye of another individual are indicated. In this case, a moult took place about three months after removal of the entire eye, yet the regeneration here is apparently not further advanced than in the preceding case. The regenerated portion shows no resemblance to an eye or any other appendage. The optic nerve still persists, for it can be seen passing into the mass of tissue on the right (*op*). No more new tissue has been formed than would equal in volume the stalk of the normal eye. It does not seem probable in this and in the preceding instance that any definite structure would have been formed, if the crayfish had lived for a much longer period.

(c.) *Theoretical Considerations.* In considering these cases of heteromorphosis, it is noteworthy that in no instance is there anything like a basal protopodite present. In every case the main structure arises from a slightly rounded eminence which is in no way similar to a protopodite. This is again different from the majority of cases cited by Herbst. In all of his figures, except one, there is shown some sort of a base from which the principal portion of the appendage arises. The base is not altogether similar to a typical protopodite, yet there is sufficient resemblance to suggest it.

Undoubtedly, as Herbst suggests, the regenerated heteromorphic structure receives a plausible explanation on the ground of reversion to an ancestral condition. The question as to whether or not the eyes are modified appendages is difficult of solution, and the study of embryology has not yielded a defi-

nite answer. Yet, from an examination of the regeneration of the eyes of crayfish, it may at least be said that there are certain reasons for regarding the eyes as modified segmental appendages, and the heteromorphic structures which arise when the entire eye is removed indicate strongly that this interpretation is the correct one.

One of the most significant points in this connection seems to me to lie in the fact that the structure which is regenerated is not always an antenna-like organ. For instance, the structure represented in Fig. 30, Pl. IV, shows no segments whatever but has very much the appearance of the bud of an ordinary appendage before constrictions appear which mark the future joints. And furthermore, the fact that the regenerated structures sometimes appear in an antenna-like form, sometimes with one, sometimes with a pair of flagella, may point to a gradual transformation of the typical appendage, the appendage in its phylogony, having passed through an antenna-stage before reaching the condition of an eye.

There may be some objection to regarding this heteromorphosis as atavism on the ground that the regenerated structure lacks a basal protopodite. This, however, might be explained on the hypothesis of partial reversion. And, if it were possible to keep the same individual alive long enough to remove successively the regenerated structures, as can be done in other regenerated appendages, there would probably finally be regenerated an appendage more nearly approaching the typical form. However, Herbst, in some of his experiments, removed the heteromorphic structure, both wholly and in part, and found that a similar structure was always regenerated. In the shrimp, Herbst found an indication of a segmented basal portion, but the segments possessed no movable articulations. Although here there was a nearer approach to an ordinary appendage than I

have found in the crayfish, still it was far from being typical. In spite of the differences in the character of the regeneration of the shrimp and crayfish, there are sufficient similarities to point to a close relationship; and both instances may be regarded as arguments in favor of considering the Decapod eye a modified segmental appendage. Morgan's experiments upon the eyes of the hermit crab should be considered as belonging to the same series. But as he gives no details, I am unable to say whether the regenerated structures in the crab are more nearly like those regenerated by the crayfish or those regenerated by the shrimp.

It is possible that, if the conditions for experiment could be rendered more natural and hence more favorable, many additional facts could be learned which would be decisive in favor of reversion or against it, but at present the question must remain an open one.

IV. GENERAL CONSIDERATIONS.

It is not my intention to enter upon a detailed, theoretical discussion of regeneration. The phenomena of regeneration are not more explicable in the crayfish than in other animals, and at present it is not theory but fact that will prove useful in the ultimate solution of the problem if a solution is ever to be reached.

The points of interests to be mentioned here are to be found in the similarities and differences between my own experiments and those of others. For instance, in Herrick's experiments upon the lobster, he comes to the conclusion that the power of regeneration is regulated according to the uses of the parts injured. He says (loc. cit., p. 107), "The power of regenerating a lost part varies in both vertebrates and invertebrates in direct proportion to the physiological importance of the part, as Weismann has clearly shown."

Later investigation has not substantiated this view. Morgan, from his experiments upon the hermit crab, which have been mentioned in an earlier part of this paper, was led to take a precisely opposite ground in regard to the relation of regenerating capacity to the physiological importance of the part concerned and its liability to injury. He found that the hermit crab was capable of regenerating any appendage that was removed, even though under natural conditions, the possibility of injury to the appendage, as in the case of the abdominal appendages which are not exposed, was practically precluded.

He also found that appendages frequently injured regenerated not only at the usual breaking joint, but were capable of regenerating from a point either above or below that level. From these observed facts he concludes that for the hermit-crab at least, there is no relation whatever between power of regeneration and liability to injury. Not only for the hermit crab, but for all other animals that are capable of regeneration, he denies the existence of any such relation.

My own observations upon the crayfish tend to substantiate Morgan's view. I have found that appendages do regenerate which under normal conditions are not usually injured, and that they are capable of regenerating from levels other than that of the usual breaking joint. In my paper on The Crayfish of Missouri (:02) I mentioned the extreme uniformity of parts in *C. gracilis,* a species which spends nearly its entire life in burrows and is therefore protected from injury.

This uniformity shows that the appendages are seldom or never injured under normal conditions. In only one instance, and that has been since the above paper was written, have I ever found an adult of *C. gracilis* that had lost an appendage. Yet I found that when young individuals of this species were used for experiment, new appendages were restored as fre-

quently and as rapidly as in *C. virilis*, a species in which injuries often occur under normal conditions. The above facts furnish additional evidence for Morgan's contention that the capacity for regeneration is independent of the physiological importance of the part or its liability to injury. It is true, however, that the physiological importance of a part bears a certain relation to the rate of regeneration, which is doubtless to be explained on the ground of differences in nutritive conditions. The greater food-supply of a part which exhibits a higher functional activity may well account for a more rapid regeneration than in the case of a part which is of less physiological importance. I have found that the thoracic appendages of the crayfish, for example, regenerate more rapidly than the swimmerets.

V. SUMMARY.

1. Experience has shown that the most favorable season of the year to experiment upon crayfish is during late winter and early spring, a short time before the moults are likely to occur; also that crayfish of small or medium size afford the most favorable material for experiment.

2. The age of the individual, the season of the year and the length of time the crayfish has been kept in confinement before being operated upon influence the results. Crayfish that have been brought in recently should always be used. The inactive life, which they necessarily lead in the laboratory, lowers their vitality and consequently reduces the chances of success.

3. Regeneration of the chelipeds normally takes place from the distal surface of the second joint; it may occur, however, from a higher level, but it has never been definitely proved that it takes place from a lower level. Normally, the appendage appears on the exterior but it may be developed within the old stump.

4. In the other thoracic appendages regeneration is similar to the same process in the chelipeds.

5. In both chelipeds and ambulatory appendages, if an injury occurs near the tip, the limb is not thrown off at the breaking joint, nor is there any apparent regeneration before a moult takes place. At the time of a moult the appendage appears restored.

6. The flagella of the antennae and antennules are readily regenerated when broken off at the base. When broken off at higher levels I have never definitely determined that regeneration occurs.

7. I have not found that the swimmerets, i. e., the second, third, fourth and fifth abdominal appendages, regenerate. Experiments, however, on these parts were not extensive.

8. In the male the first pair of abdominal appendages regenerate. These appendages are modified as accessory reproductive organs.

9. The telson and also the sixth pair of abdominal appendages, which with the telson form the tail-fin, are regenerated.

10. Parts of the exoskeleton and the gills were experimented upon without definite results.

11. When only the distal part of the eye is removed an apparent regeneration takes place in a very short time. Examination, however, shows that true functional regeneration has not occurred. The ommatidia are not regenerated and in only one instance have I found any indication at all that the corneal facets were being restored. The eye usually develops in an irregular, abnormal form, the irregularity sometimes being due to the unequal development of the cuticle and sometimes to the tissues beneath.

12. When the whole eye is removed regeneration may or may not take place. If any structure is regenerated it is not

an eye but either a short appendage that consists of one, two, or three segments, or a longer structure consisting of either one or a pair of antenna-like flagella.

13. Before any regenerated part can assume a normal appearance it is necessary that a moult take place. Before a moult the regenerated portions are much compressed and when released may expand to twice the proportions permitted by the confining cuticle.

14. The general results of this series of experiments substantiate the view that the power of regeneration is independent of the liability of the part to injury. The results also show that the rate of regeneration depends both upon external factors and the physiological condition of the animal operated upon.

Zoölogical Laboratory,
University of Missouri,
June 1, 1901.

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EXPLANATION OF PLATES

REFERENCE LETTERS:

a, b, c, etc. refer to particular topographical regions of figures described in the text.

an.	antennule.
at.	antennae.
ba.	bacteria.
b. m.	basal membrane.
ca.	carapace.
cc.	connective tissue.
c. f.	corneal facets.
cr.	crystalline cones.
cu.	cuticle.
g.	granules.
hy.	hypodermis.
m.	muscle fibers.
n.	regenerated portion.
n. c.	new cuticle.
o. c.	old cuticle.
om.	ommatidia.
p.	pigment.

PLATE 1

Figs. 1, 2, 3, and 4. Preliminary stages in regeneration of new chela.

Fig. 1. Smooth stump before regeneration appears externally.

Fig. 2. First appearance of bud.

Fig. 3. Constrictions marking segments have appeared.

Fig. 4. Rudiments of the entire regenerated structure laid down.

Fig. 9. Regeneration that has taken place within the old stump, behind the cuticle stretched across the wounded surface. *a*, the old stump; *b*, the regenerated portion.

Fig. 10. Same as *b* in Fig. 9, after removal of an investing membrane.

Fig. 11. Section through regenerated structure shown in Figs. 9 and 10.

Fig. 14 and 15. Regenerating 6th abdominal appendage before and after a moult respectively.

Figs. 16 and 17. Regenerating telson, dorsal and ventral views.

Fig. 18. Regenerating flagellum of right antenna, dorsal view.

Fig. 19. Leg regenerating from distal end of 3rd joint. Regenerated portion 3 days old.

Fig. 20. Double chela. Meros branched.

Fig. 38. Hypodermis from end of developing chela, 5 days old.

Fig. 39. Developing muscle fibers from central portion of regenerating chela, bud 5 days old.

Fig. 40. Developing muscle fibers from chela bud older than shown in Fig. 39, but not yet constricted into segments.

PLATE 2

Fig. 5. Cast-off shell of crayfish, indicating the number of appendages that had been broken off.

Fig. 6. Crayfish that has just moulted from shell shown in Fig. 5. Left chela broken off in moulting.

Fig. 7. Cast off shell of crayfish showing visible regeneration before a moult.

Fig. 8. Crayfish that has moulted from shell shown in Fig. 7.

Fig. 12. Developing muscular tissue from region *e* (Fig. 11). Fibers very loose and granular with large, deeply staining nuclei.

Fig. 13. Developing muscular tissue from region *a* (Fig. 11). Two bands consisting of two and four fibers respectively, separated by a broad granular space.

Fig. 36. Section of regenerating eye shown in Fig. 35. Specimen killed just after a moult.

Fig. 37. Section of same eye as Fig. 36, but more eccentric.

PLATE 3

Fig. 21. Regenerating eye. Corneal portion had been removed 44 days before. Lamellae of new cuticle very loose and very irregularly developed—extends from *a* to *b*.

Fig. 22. Regenerating eye. Corneal portion had been removed 33 days before. Outline of regenerated part very irregular, due to unequal development of connective tissue beneath newly formed cuticle. *ba.* probably bacteria that have become massed over the irregular surface ot the new cuticle.

Fig. 23. Cuticular portion of regenerating eye from which one side of cornea had been removed 10 days before. At upper left hand portion of figure the remainder of old corneal facets are being thrown off. The hypodermis is a continuous, wel l defined structure beneath the whole cuticle.

Fig. 24. Section of regenerating eye, corneal portion removed 44 days before. Section taken eccentrically. The old cuticle covers most of the surface, the new cuticle developing beneath. No well defined hypodermis.

Fig. 25. Section from same eye as Fig. 24, but less eccentrically taken. New cuticle much better developed in this region; large space between new and old cuticle.

Fig. 26a. Section through regenerating eye. Cornea had been removed 24 days. A remnant of old crystalline cones shown at *cr*. Degenerating optic ganglion (gl) on right side. Regenerating muscle fibers extending to top of eye.

PLATE 4

Fig. 26. Eye from which almost entire cornea was removed Apr. 9. Moulted and was killed, May 3.

Fig. 27. Entire right eye removed. Flagella (*aa*) appeared in three months, after crayfish had moulted twice.

Fig. 28. Normal eye and regenerated growth from a crayfish that had had entire eye removed four months before. No moult had taken place.

Fig. 29. Normal eve and regenerated growth from a crayfish whose eye was removed three months before, at end of which time a moult took place.

Fig. 30. Stump of old eye-stalk and regenerated growth that developed between May 9, the date of removal of the eye, and Aug. 20 when individual died. It had moulted twice in the interval.

Fig. 31. Side view of anterior portion of crayfish, rostrum dissected away. This structure developed between June 9 and Aug 20. A moult occurred Aug 20.

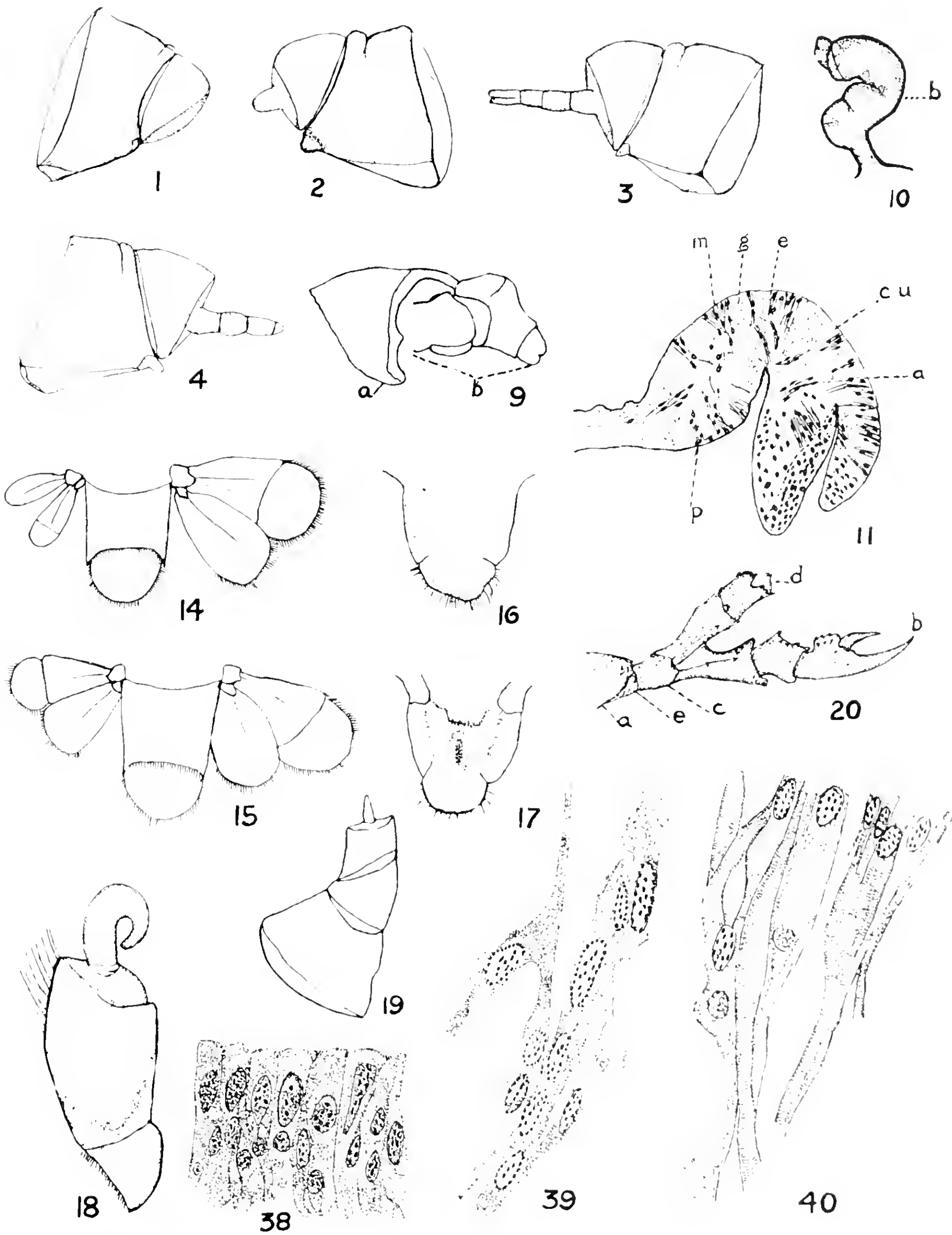
Fig. 32. Another crayfish, shown as in Fig. 31, except rostrum has not been removed.

Fig. 33. The same as Fig. 31, except that in this specimen left eye instate of right had been removed, and the regenerated structure is composed of a greater number of segments. Regeneration shown has taken place in two months, one moult having occurred.

Fig. 34. Similar to the preceding.

Fig. 35. Normal eye and regenerating eye six weeks after removal of entire cornea, one moult having taken place.

Fig. 41. Muscle fibers taken from *a*, Fig. 26a.



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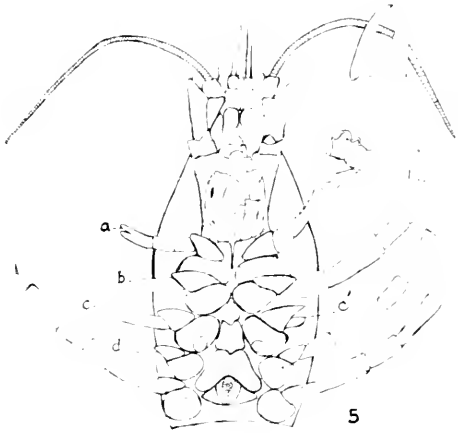
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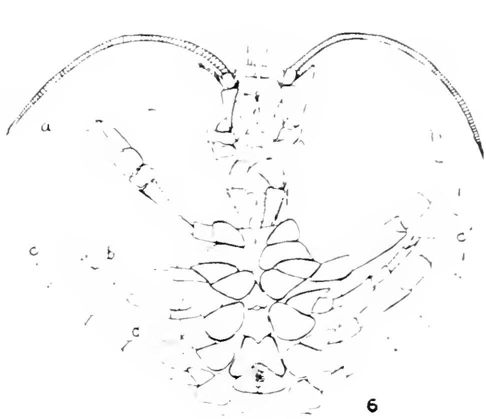
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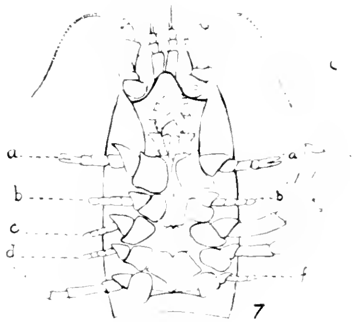
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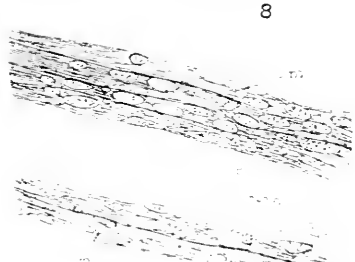
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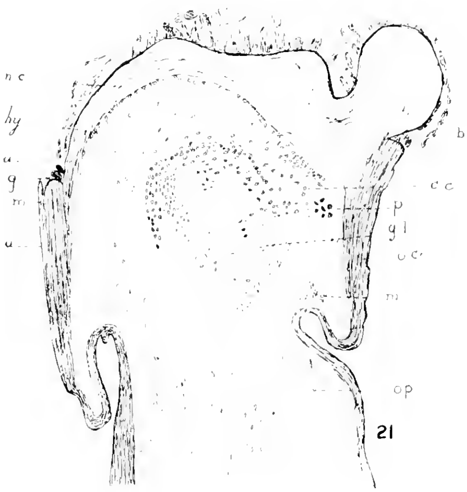

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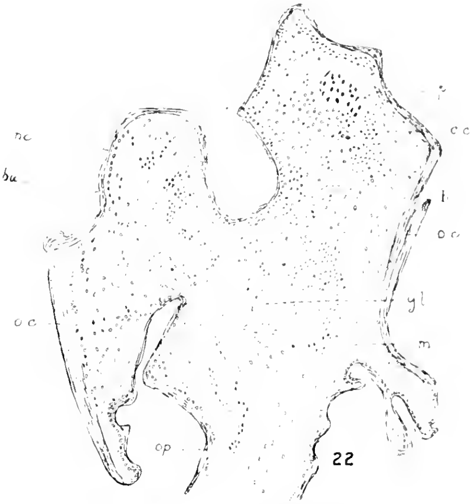

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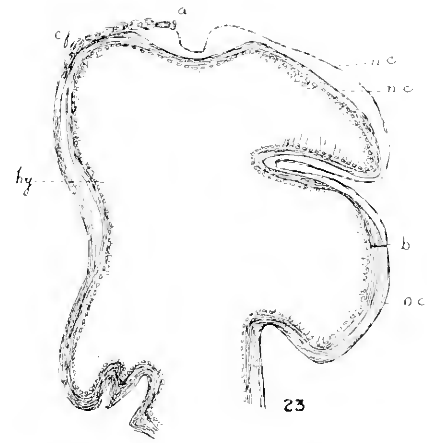

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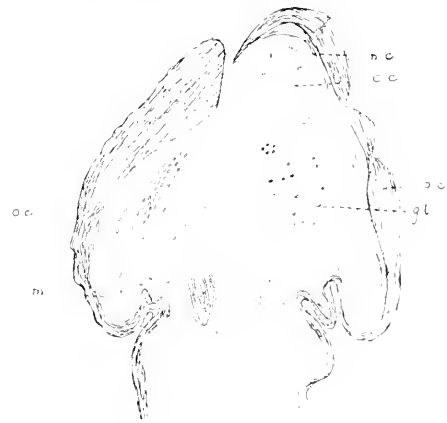

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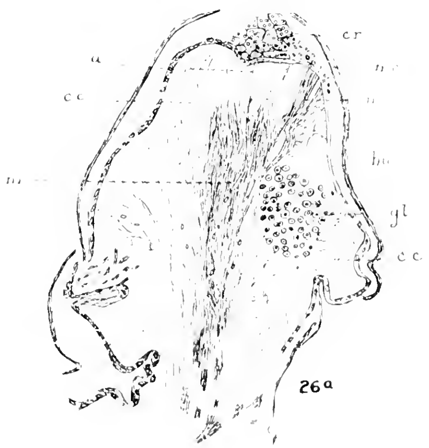

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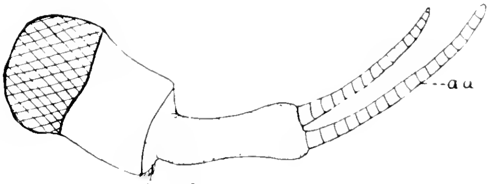

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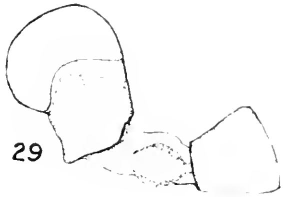

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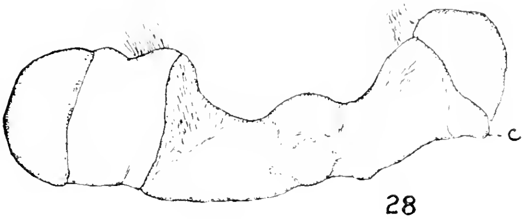

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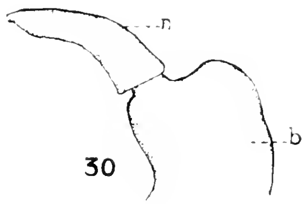

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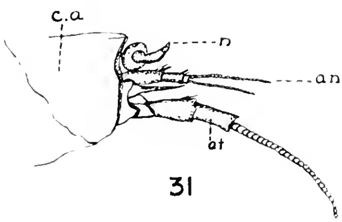

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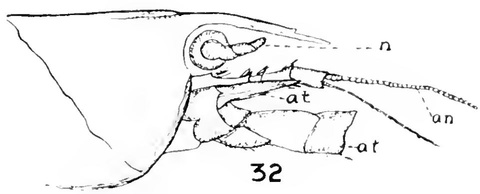

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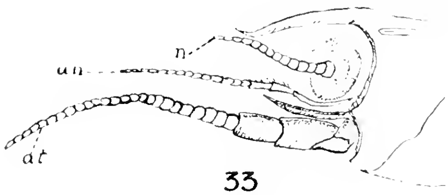

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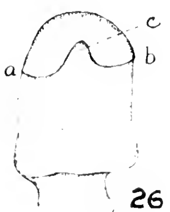

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